1. Advanced Profile

Other Required Profile Information

Immigration (status/visa) :	
Current Academic Title :	
Current Academic Position :	
Career Stage :	

Institution Where Work Will Be Done

Institution :	Washington University, School of Medicine
Department :	
Division :	

Professional Time Usage Details

Administration :	0%
Patient Care :	0%
Research :	0%
Teaching :	0%
Coursework :	0%
Other :	0%
Total :	0%

2. Project Summary

Application Info

Application ID :	18PRE33960417
Funding Component Name :	Association Wide
Program Name :	Summer 2018 Predoctoral Fellowship
Principal Investigator Name :	Alexander Polino
Status :	Submitted to GO
Award Start Date :	07-01-2018
Project Title :	Elucidating the role of Plasmodium N-terminal acetyltransferases in export of effectors into the host erythrocyte
Application Deadline date :	11-01-2017
Award End Date :	06-30-2020

Project Summary Details

Percent of Applicant's total effort devoted to this project :	50
Project Name :	Elucidating the role of Plasmodium N-terminal acetyltransferases in export of effectors into the host erythrocyte

Project Summary

The malaria parasite P. falciparum radically transforms its host red blood cell (RBC), exporting hundreds of effector proteins into the RBC. These exported effectors play various roles in the RBC, modifying its rigidity, solute permeability, and adhesive properties. To effect this program of protein export, the parasite must sort exported proteins from those retained in the parasite. The primary sorting signal is a motif called the Plasmodium export element (PEXEL) which is recognized and cleaved in the parasite ER. Following cleavage, the new N-terminus is acetylated; however, the contribution of acetylation to export-competence and the identity of any N-terminal acetyltransferases (NATs) involved is unknown. Previous work has shown that mutations that interfere with acetylation also block export, suggesting a possible link between these two processes. Recently, we have identified a candidate NAT that localizes to the ER, making this the first known ER-resident NAT in P. falciparum. We hypothesize that this, and potentially other ER-resident NATs, acetylate exported proteins, and that this modification is essential for effective protein export. We will test this hypothesis with the following aims:
(1) Identify ER-resident NAT(s) - Sequence analysis reveals six candidate NATs. We have used immunofluorescence (IF) assays to localize one candidate to the ER; however, the remaining five are uncharacterized. Here we will use CRISPR-Cas9/Cpf1 mediated genomic editing to tag each NAT with the fluorescent protein mNeonGreen. We will use fluorescent microscopy to assess colocalization of the NATs with the ER marker BiP. NATs that localize to the ER will be further investigated in Aim 2, while NATs that localize elsewhere will be reported to support further study into Plasmodium NATs.
(2) Assess the role of ER-resident NAT(s) in protein export - We have recently used IF microscopy to show that Pf3D7_1437000 localizes to the ER. Here, we will use the TetR-DOZI regulated knockdown system to interrogate the function of Pf3D7_1437000 and other ER-localized NATs from Aim 1. The effect of NAT depletion on protein export will be assessed by IF using antibodies against exported proteins. Additionally, the effect of knockdown on acetylation of exported proteins will be assessed by tandem mass spectrometry. If our hypothesis is correct, we expect to see that depletion of ER-resident NAT(s) leads to decreased acetylation of exported proteins and decreased protein export.

3. Science Classification

Research Classification Type

Research Classification Type:	Basic Research is meant to increase our scientific knowledge base by studying fundamental life processes. This type of research is aimed at increasing our understanding of basic biological, behavioral or disease mechanisms. While Basic Research may ultimately lead to treatments or therapies, the goal of Basic Research studies is to establish a proof of principle. Research that exclusively involves in vitro studies using human tissues not linked to identified individuals falls within Basic Research.
Is the project translational?	No

Science Classification

Major Science Classification(1)	Sub-Classification(s)
Microbiology and Microbial Pathogenesis - Basic Science	1. Microbial Molecular Genetics 2. Parasitic Infections 3. Pathogenicity and Virulence Factors
Major Science Classification(2)	Sub-Classification(s)
Basic Cell - Membranes and Subcellular Organelles	1. Endoplasmic Reticulum Function 2. Intracellular Trafficking

4. Research Classification

Research Classification 1

CVD/Stroke Classification :	Broadly Stroke-Related

5. Institutional Personnel

Grants Officer 1

Position Title :	
First Name :	Teri
Last Name :	Medley
Phone Number :	
E-mail :	researchgrants@wusm.wustl.edu

Fiscal Officer 1

Position Title :	
First Name :	Teri
Last Name :	Medley
Phone Number :	
E-mail :	researchgrants@wusm.wustl.edu

6. Third Party Personnel

Sponsor 1

Degree :	MD PhD
Institution Name :	Washington University, School of Medicine
Position Title :	Professor
First Name :	Daniel
Last Name :	Goldberg
Phone Number :	3143621514
E-mail :	dgoldberg@wustl.edu

Referent 1

Degree :	
Institution Name :	Cornell Univeristy College of Veterinary Medicine
Position Title :	Associate Professor of Virology
First Name :	John
Last Name :	Parker
Phone Number :	
E-mail :	jsp7@cornell.edu

Referent 2

Degree :	
Institution Name :	Washington University School of Medicine
Position Title :	Professor
First Name :	David
Last Name :	Sibley
Phone Number :	

E-mail :	sibley@wustl.edu

Referent 3

Degree :	
Institution Name :	Washington University School of Medicine
Position Title :	Associate Professor
First Name :	James
Last Name :	Fleckenstein
Phone Number :	
E-mail :	jflecken@wustl.edu

7. Lay Summary Form

Research Categories

Research Name :	1. Genetics 2. Basic biomedical research

Lay Summary Form

1. What is the major problem being addressed by this study? :	While we know the malaria parasite sends hundreds of proteins into the host red blood cell, we do not know how the parasite decides which proteins to export and which to retain in its own cell. When making proteins to send out into the host cell, the parasite modifies them by adding a small chemical tag called an acetyl group. Mutated parasite proteins that cannot have this acetyl group added, also cannot be exported into the host cell, suggesting this modification plays a critical role in determining which proteins should be sent into the host cell. Here we will identify the parasite machinery responsible for this chemical modification and determine whether this machinery (and therefore the addition of this acetyl group) is required for export of proteins into the host cell.

2. What specific questions are you asking and how will you attempt to answer them?	1. Which parasite proteins are in the right place to be involved? We know that this modification occurs in a parasite structure called the endoplasmic reticulum (ER) and that it must be done by a certain kind of protein (called an ¿acetylase¿). To determine which acetylases may be involved, we will use genetic manipulation to add a visible tag to each, then use microscopy to determine which acetylases are in the parasite ER. 2. Are acetylases important for exporting proteins into the host cell? Here we will genetically suppress each acetylase in the ER. We will then test if suppressing a given acetylase causes the parasite to fail to add acetyl groups to exported proteins, and what effect this has on the ability of the parasite to send proteins into the host cell.
3. Overall, what is the potential impact of this work to the mission of the AHA? You might address: What is the long-term biomedical significance of your work, particularly as it pertains to the cardiovascular area? What major therapeutic advance(s) do you anticipate that it will lead to? For instance, new drug(s), a surgical technique/procedure, a diagnostic tool/test, a previously undetected risk factor, etc :	Malaria infects 212 million people each year, resulting in over 400,000 deaths. The majority of these are in children under 5 years and are caused by blockage of blood vessels in the brain, termed ¿cerebral malaria¿. Those that survive are at risk for negative cardiovascular outcomes later in life due to blood vessel damage and inflammation suffered during infection. Our proposed work investigates the mechanisms by which the malaria parasite successfully infects red blood cells and causes disease. A deeper understanding of this process may uncover new targets for antimalarial drugs, and will expand our knowledge of how this parasite functions within red blood cells.

8. Budget

Expense Type Name :	Fringe
Yearly Fund :	1000.0, 1000.0
Expense Type Name :	Salary
Yearly Fund :	23844.0, 23844.0
Expense Type Name :	Project Support
Yearly Fund :	2000.0, 2000.0
Total :	53688.00

9. Funds Available

Funds Available Details

Do you have any active, pending, or approved awards?	true

Funds Available 1

Funding Agency :	NIH
Award ID Number :	5T32GM007067
Project Title :	Washington University Cell & Molecular Biology Training Grant
Principal Investigator :	James Skeath
Applicants Role :	Trainee
Start Date :	09-01-2016
End Date :	08-31-2018
Award Status :	Active
Total Amount :	47220.00
Amount Available to Applicant :	47220.00
Annual Amount :	0.00
Is this project overlapping (current award) or alternative (pending award) to this AHA proposed project?	yes

10. Research Subjects / Assurances

Endorsement 1

Description :	Adult Human Stem Cells
Answer :	no
DocumentName :	

Endorsement 2

Description :	Biohazards (other than Recombinant DNA)
Answer :	yes
DocumentName :	

Endorsement 3

Description :	Cloning
Answer :	no
DocumentName :	

Endorsement 4

Description :	Human Subjects
Answer :	no
DocumentName :	

Endorsement 5

Description :	Recombinant DNA
Answer :	yes
DocumentName :	

Endorsement 6

Description :	Human Embryonic or Fetal Stem Cells
Answer :	no
DocumentName :	

Endorsement 7

Description :	Human Fetal Tissue
Answer :	no
DocumentName :	

Endorsement 8

Description :	Vertebrate Animal Subject
Answer :	no
DocumentName :	

11. Science and Evaluation Information

11.1. Applicant's Biographical Sketch

Applicant's Biographical Sketch - APolino Biosketch.pdf

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Polino, Alexander

eRA COMMONS USER NAME (credential, e.g., agency login): ajpolino

POSITION TITLE: Research Assistant

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	START DATE MM/YYYY	END DATE MM/YYYY	FIELD OF STUDY
Cornell University, Ithaca, New York	BA	08/2011	05/2015	Biological Sciences
Washington University School of Medicine, St Louis, Missouri	PHD	08/2015	05/2020	Molecular Microbiology

A. Personal Statement

When I entered college at Cornell University, the incredible breadth of microbiology courses offered to me quickly sparked an interest in microbiological research. To develop that interest, I joined the laboratory of Dr. John Parker at the Baker Institute for Animal Health (Cornell College of Veterinary Medicine) studying cellular stress responses to mammalian orthoreovirus infection. Through the mentoring of Dr. Parker and a postdoctoral researcher in the lab, I began to develop technical laboratory skills, as well as an appreciation for the challenges of molecular biology research, and the mindful troubleshooting required to accomplish project goals. This work culminated in an honors thesis exploring the role of eIF2-alpha phosphorylation in cell defense against different virus strains. To further pursue my interest in microbial pathogenesis, I enrolled in graduate school at Washington University School of Medicine where I joined the laboratory of Dr. Daniel Goldberg, an established investigator in the field of malaria research with a record of training successful scientists. At Washington University, I have found a dynamic training environment, with stimulating seminars, thoughtful and enthusiastic peers, and plentiful opportunities for interaction with faculty. In the Goldberg lab, I am using novel genetic tools to interrogate the functions of genes which just a few years ago were recalcitrant to genetic study. I'm currently investigating how the parasite marks certain proteins for export into the erythrocyte, while ensuring that others are maintained within the parasite. The Goldberg lab provides an environment with high expectations, but incredible support both in terms of laboratory resources and helpful colleagues. In this environment and with Dr. Goldberg's guidance, I expect to develop the mindset and skills required to pursue a career in research science, with the long-term career goal of obtaining a university faculty position.

B. Positions and Honors

Positions and Employment

2013 - 2015 Research Assistant, Cornell University, Baker Institute for Animal Health, Ithaca, NY

Other Experience and Professional Memberships

Honors

2014 - 2015 Pauline and Irving Tanner Dean's Scholar Program, Cornell University

2016 - 2017 Victoria A. Fraser, M.D. Fellowship, Washington University School of Medicine, Dept. of Medicine

Applicant's Biographical Sketch - APolino Biosketch.pdf

2017 Best Poster Award, 28th Molecular Parasitology Meeting
2017 - 2019 Microbiology Program Steering Committee, Student Representative

C. Contribution to Science

1. As an undergraduate, I spent two years in the laboratory of Dr. John Parker investigating cellular stress responses to mammalian orthoreovirus (MOR) infection. Other groups had previously observed eIF2-alpha phosphorylation (a key marker of cell stress) in some MOR strains but not others. I designed and executed time course analyses to show that infection with all known strains of MOR results in eIF2-alpha phosphorylation, though the timing differs substantially (resulting in phosphorylation being missed in some previous literature). Interested in how the virus could escape this potent host stress response, I performed immunofluorescence assays and showed that eIF2 is heavily recruited to sites of virus replication, but the phosphorylated form is excluded, suggesting MOR may construct a replication-permissive niche inside an otherwise stressed cell. My work in the Parker lab was funded in part by the Pauline and Irving Tanner Dean's Scholar Program at Cornell University, and culminated in my undergraduate research thesis as well as a poster presented internally at a university event.

2. My current research in Dr. Daniel Goldberg's lab is focused on early events that guide the trafficking of exported effectors into the host erythrocyte. I have used a recently-described knockdown method to make the first lethal knockdown of the ER-resident protease plasmepsin V (PMV) which recognizes and cleaves a key motif present in most exported proteins. This knockdown validated PMV as a potential drug target, and showed an unexpected phenotype distinct from disruptions of other export components, suggesting a critical export-independent role for PMV in addition to its validated role in protein export. This work was the subject of my short talk and poster at the recent 28th Molecular Parasitology Meeting at Woods Hole, MA for which I was awarded a Best Poster Award. This work is in preparation for submission to for publication by the end of 2017. Additionally, I have begun work to identify N-terminal acetyltransferases that acetylate known exported proteins, and assess their function. Preliminary work on this project is outlined in the attached Research Proposal.

    a. Polino AJ, Nasamu S, Istvan E, Niles J, Goldberg DE. Assessment of biological role and insight into druggability of the *P. falciparum* aspartic protease plasmepsin V. 28th Annual Molecular Parasitology Meeting. Woods Hole, MA. Sept. 2017 (talk/poster).

D. Additional Information: Research Support and/or Scholastic Performance

Scholastic Performance

YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
CORNELL UNIVERSITY					
2011	Honors General & Inorganic Chem.	B+	2011	Beginning Mandarin Chinese I	A-
2012	Intro Comparative Physiology	A-	2011	Honors Calculus II	A-
2012	Honors General & Inorganic Chem. II	B+	2011	Writing Seminar: Short Stories	A
2012	Intro Evolutionary Biology & Diversity	A-	2012	China Under Revolution & Reform	A-
2012	Investigative Biology Lab	A-	2012	Beginning Mandarin Chinese II	A-
2012	Honors Organic Chem. I	C+	2012	Writing Seminar: Marx, Nietzsche, Freud	A
2013	Intro Cell Biology & Development	B-	2012	Object-Oriented Programming & Data Structures	B
2013	Principles of Biochemistry	A-	2012	Linear Algebra	B

Applicant's Biographical Sketch - APolino Biosketch.pdf

2013	Computer Graphics & Molecular Biology	A	2014	Ethical Issues in Health & Medicine	A
2013	Intro to Neuroscience	S	2014	Intro to the History of Medicine	B+
2013	Intro to Experimental Organic Chem.	A	2014	Careers in Research	A
2013	Organic Chem. for the Life Sciences	B+	2014	Teaching Experience: Principles of Genetics	A+
2013	General Microbiology	A	2015	Intro to Southeast Asia	S
2013	General Microbiology Laboratory	B+	2015	Teaching Experience: Pathogenic Bacteriology	A
2013	Medical Parasitology	A			
2013	Basic Immunology	A-			
2013	Drugs & the Brain	A-			
2013	Statistics & Research Design	A			
2014	Genetics & Genomics Lecture	B			
2014	Genetics & Genomics Laboratory	B+			
2014	Survey of Cell Biology	B+			
2014	Laboratory in Biochem. & Molecular Biology	A-			
2014	Pathogenic Bacteriology	A-			
2014	Paleoparasitology	A			
2014	Independent Undergraduate Research	A			
2014	Cell Proliferation, Senescence, & Death	A+			
2014	Senior Seminar in Microbiology	S			
2014	Principles of Virology	A			
2015	Independent Undergraduate Research	A+			
2015	Cellular Microbiology	B+			
WASHINGTON UNIVERSITY SCHOOL OF MEDICINE					
2015	Fundamentals of Molecular Cell Bio.	A-	2017	Ethics and Research Science	S
2015	Fundamentals of Biostatistics	A			
2015	Nucleic Acids & Protein Biosynthesis	A-			
2016	Molecular Microbiology & Pathogenesis	B			
2016	Special Topics in Microbial Pathogenesis	A-			
2017	Molecular, Cell and Organ Systems	A+			
2017	Translational Aspects of Infectious Diseases Research	S			

Classes marked as "S" were marked only as "Satisfactory" (S) or "Unsatisfactory" (U)

11.2. Academic Record (Predoc)

Academic Record (Predoc) - See Biosketch.pdf

See Biosketch

11.3. Literature Cited

Literature Cited - APolino Literature Cited.pdf

Literature Cited:

1. World Health Organization: *2016 World Malaria Report*. World Health Organization; 2016.
2. Celermajer DS, Chow CK, Marijon E, Anstey NM, Woo KS: **Cardiovascular Disease in the Developing World**. *J. Am. Coll. Cardiol.* 2012, **60**:1207–1216.
3. Moxon CA, Chisala N V., Wassmer SC, Taylor TE, Seydel KB, Molyneux ME, Faragher B, Kennedy N, Toh C-H, Craig AG, et al.: **Persistent Endothelial Activation and Inflammation After Plasmodium falciparum Infection in Malawian Children**. *J. Infect. Dis.* 2014, **209**:610–615.
4. Chang HH, Falick AM, Carlton PM, Sedat JW, DeRisi JL, Marletta MA: **N-terminal processing of proteins exported by malaria parasites**. *Mol. Biochem. Parasitol.* 2008, **160**:107–115.
5. Beck JR, Muralidharan V, Oksman A, Goldberg DE: **PTEX component HSP101 mediates export of diverse malaria effectors into host erythrocytes**. *Nature* 2014, **511**:592–595.
6. Elsworth B, Matthews K, Nie CQ, Kalanon M, Charnaud SC, Sanders PR, Chisholm SA, Counihan NA, Shaw PJ, Pino P, et al.: **PTEX is an essential nexus for protein export in malaria parasites**. *Nature* 2014, **511**:587–91.
7. Marti M, Good RT, Rug M, Knuepfer E, Cowman AF: **Targeting malaria virulence and remodeling proteins to the host erythrocyte**. *Science* 2004, **306**:1930–3.
8. Grüring C, Heiber A, Kruse F, Flemming S, Franci G, Colombo SF, Fasana E, Schoeler H, Borgese N, Stunnenberg HG, et al.: **Uncovering Common Principles in Protein Export of Malaria Parasites**. *Cell Host Microbe* 2012, **12**:717–729.
9. Tarr SJ, Cryar A, Thalassinos K, Haldar K, Osborne AR: **The C-terminal portion of the cleaved HT motif is necessary and sufficient to mediate export of proteins from the malaria parasite into its host cell**. *Mol. Microbiol.* 2013, **87**:835–850.
10. Boddey JA, Carvalho TG, Hodder AN, Sargeant TJ, Sleebs BE, Marapana D, Lopaticki S, Nebl T, Cowman AF: **Role of Plasmepsin V in Export of Diverse Protein Families from the Plasmodium falciparum Exportome**. *Traffic* 2013, **14**:532–550.
11. Monda JK, Scott DC, Miller DJ, Lydeard J, King D, Harper JW, Bennett EJ, Schulman BA: **Structural Conservation of Distinctive N-terminal Acetylation-Dependent Interactions across a Family of Mammalian NEDD8 Ligation Enzymes**. *Structure* 2013, **21**:42–53.
12. Behnia R, Panic B, Whyte JRC, Munro S: **Targeting of the Arf-like GTPase Arl3p to the Golgi requires N-terminal acetylation and the membrane protein Sys1p**. *Nat. Cell Biol.* 2004, **6**:405–413.
13. Spillman NJ, Beck JR, Ganesan SM, Niles JC, Goldberg DE: **The chaperonin TRiC forms an oligomeric complex in the malaria parasite cytosol**. *Cell. Microbiol.* 2017, **19**:e12719.
14. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A, et al.: **Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system**. *Cell* 2015, **163**:759–71.
15. Shaner NC, Lambert GG, Chammas A, Ni Y, Cranfill PJ, Baird MA, Sell BR, Allen JR, Day RN, Israelsson M, et al.: **A bright monomeric green fluorescent protein derived from Branchiostoma lanceolatum**. *Nat. Methods* 2013, **10**:407–409.
16. Ganesan SM, Falla A, Goldfless SJ, Nasamu AS, Niles JC: **Synthetic RNA–protein modules integrated with native translation mechanisms to control gene expression in malaria parasites**. *Nat. Commun.* 2016, **7**:10727.
17. Ross PL, Huang YN, Marchese JN, Williamson B, Parker K, Hattan S, Khainovski N,

Literature Cited - APolino Literature Cited.pdf

Pillai S, Dey S, Daniels S, et al.: **Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents**. *Mol. Cell. Proteomics* 2004, **3**:1154–69.

11.4. Applicant's Publication 1

Applicant's Publication 1 - MPM 2017 Abstract Final.pdf

Assessment of biological role and insight into druggability of the *Plasmodium falciparum* aspartic protease plasmepsin V

Polino AJ[1], Nasamu AS[1], Istvan EI[1], Niles JC[2], Goldberg DE[1#]

[1]Washington University School of Medicine

[2]Massachusetts Institute of Technology

[#]corresponding author

The aspartic protease plasmepsin V (PMV) is considered a promising drug target due to its critical role in export of *Plasmodium* effector proteins into the host erythrocyte. While PMV has long been presumed essential, genetic validation is incomplete and study of PMV has been hindered by the apparent resilience of parasites to substantial PMV depletion. Ten-fold reduction of PMV protein levels has no effect on parasite growth or protein export. We report construction of a TetR-aptamer-controlled parasite line that allows tighter regulation of PMV levels. Surprisingly, knockdown of PMV resulted in parasite death immediately after invasion. This is distinct from the phenotype described for knockdown of PTEX translocon components involved in protein export, where parasites develop through the ring stage and arrest at the ring-trophozoite transition. The data suggest that PMV plays an unappreciated role early in the intraerythrocytic development cycle independent of protein export. Anhydrotetracycline titration allowed tunable PMV expression and revealed that PMV levels must be reduced >99% to restrict parasite growth. We conclude that PMV is made in great excess relative to amounts needed for homeostasis in cultured parasites. These findings could have important implications for antimalarial drug discovery efforts aimed at targeting PMV function in parasites.

11.5. Applicant's Publication 2

Applicant's Publication 2 - Not Available.pdf

Not Available

11.6. Applicant's Publication 3

Applicant's Publication 3 - Not Available.pdf

Not Available

11.7. Research Plan 5 Pages

Research Plan 5 Pages - APolino Research Plan.pdf

Elucidating the role of *Plasmodium* N-terminal acetyltransferases in export of effectors into the host erythrocyte

1. Specific Aims

The malaria parasite *Plasmodium falciparum* radically transforms its host red blood cell (RBC), exporting hundreds of effector proteins into the erythrocyte cytoplasm. These exported effectors play various roles in the RBC: enhancing rigidity and solute permeability, as well as studding the RBC surface with adhesins that mediate binding to vascular endothelia. To effect this program of protein export, the parasite must sort exported proteins from those meant to be retained in the parasite. The primary sorting signal is an export motif called the *Plasmodium* export element (PEXEL) which is recognized and cleaved in the parasite ER. Following cleavage, the new N-terminus is acetylated; however, the contribution of this modification to export-competence and the identity of any N-terminal acetyltransferases (NATs) involved is unknown. Previous work has shown that mutations in the two residues following PEXEL cleavage interferes with both acetylation and export, suggesting a possible link between these two processes. Study of the role of acetylation in protein export has been hindered by a lack of identified ER-resident NATs as well as poor genetic tools for studying unknown proteins. Recently, we have identified a candidate NAT which appears to localize to the ER, making this the first known ER-resident NAT in *P. falciparum*. We hypothesize that this, and potentially other ER-resident NATs, acetylate exported proteins, and that this modification is essential for effective protein export. We will test this hypothesis with the following aims:

Aim 1: Identify ER-resident NAT(s)

Sequence analysis reveals six candidate NATs transcribed during infection of RBCs. We have recently used immunofluorescence assays to localize one candidate NAT (Pf3D7_1437000) to the ER; however, the remaining five are entirely uncharacterized. Here we will use CRISPR-Cas9/Cpf1 mediated genomic editing to tag each candidate NAT with the fluorescent protein mNeonGreen. We will use fluorescent microscopy to assess colocalization of the NATs with the ER marker BiP tagged with mRuby3. NATs that localize to the ER will be further investigated in Aim 2, while NATs that localize elsewhere will be reported to stimulate further study into acetylation in *P. falciparum*.

Aim 2: Assess the role of ER-resident NAT(s) in protein export

We have recently used immunofluorescence microscopy to show that Pf3D7_1437000 colocalizes with the ER-resident protease plasmepsin V. Here, we will use the recently-described TetR-DOZI regulated knockdown system to interrogate the function of Pf3D7_1437000 as well as any other NATs found to localize to the ER in Aim 1. In each case, the effect of NAT depletion on protein export will be assessed by immunofluorescence using antibodies against exported proteins. Additionally, the effect of knockdown on acetylation of exported proteins will be assessed by tandem mass spectrometry. If our hypothesis is correct, we expect to see that depletion of ER-resident NAT(s) leads to decreased acetylation of exported proteins and decreased protein export.

2. Background and Significance

Malaria remains a devastating disease throughout the developing world, causing over 400,000 annual deaths; nearly all deaths are caused by the species *P. falciparum* [1]. Clinical disease results from infection of host RBCs, which adhere to vascular endothelia to avoid splenic clearance. This adhesion results in activation of vascular endothelia, inflammation, and in severe cases vascular obstruction and death [2]. Resolved infections have been proposed to lead to persistent inflammation, predisposing infected individuals to future cardiovascular disease [3].

When *P. falciparum* infects a RBC, it radically transforms the host cell by exporting hundreds of proteins across the parasitophorous vacuolar (PV) membrane in which the parasite is enclosed and into the RBC cytosol. This export program causes various changes to the host cell: enhancing permeability to solutes, increasing RBC rigidity, and trafficking adhesins to the RBC surface. While export of effectors across the PV membrane is essential for parasite survival, the signaling information that guides export-destined proteins into this pathway remains unclear.

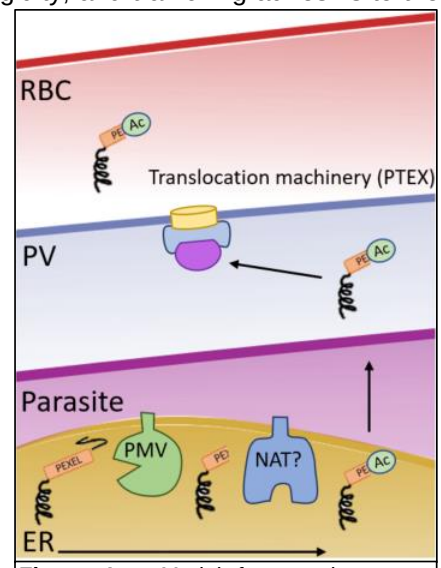

Figure 1 – Model for protein export. Exported proteins are cleaved at PEXEL by plasmepsin V (PMV) then acetylated by an unknown NAT before being translocated across the PV membrane and into the RBC cytosol.

The largest group of exported proteins contains a pentameric amino acid motif called the *Plasmodium* export element (PEXEL). PEXEL proteins are generally targeted to the ER by a N-terminal hydrophobic signal sequence. In the ER, the PEXEL sequence (RxLxE/Q/D) is recognized and cleaved after the conserved leucine, leaving a sequence of xE/Q/D. The new N-terminus is acetylated in the ER by unknown N-acetyltransferases (NATs; **Fig. 1**) [4]. The cleaved, acetylated protein is then secreted into the PV lumen, where the parasite-encoded translocation machinery threads it across the PV membrane and into the RBC cytosol [5,6].

Not all vacuolar proteins are exported into the RBC, indicating the parasite has a method to discriminate between export-destined proteins (after PEXEL cleavage) and those meant to be retained in the vacuole. Following PEXEL cleavage, the 15 most N-terminal amino acids are sufficient to drive export of GFP into the RBC, suggesting that all necessary information for export is in this 15 amino acid stretch [7]. This remains true if the cleavage event is carried out by a exogenous protease rather than the endogenous aspartic protease plasmepsin V (PMV), suggesting there is no physical "hand-off" between PMV and an interacting partner [8,9]. Of these first 15 amino acids, the only clearly restricted residues are P1' and P2' (**Fig. 2**) [7]. Mutations in these first two residues have been shown to block export without blocking PMV cleavage, suggesting these residues mediate a critical step in protein export downstream of PMV cleavage [9]. The other 13 residues can be broadly substituted or even replaced with all alanines and still support export [10]. Intriguingly, mutations in P1' and P2' that block export also block acetylation, suggesting a possible linkage between these two processes [9].

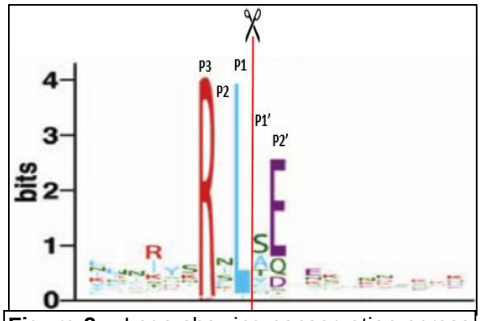

Figure 2 – Logo showing conservation across PEXEL and surrounding residues. From [7].

In other systems, N-terminal acetylation (NtA) plays various roles in functional protein-protein interactions [11] or proper protein localization [12]; however, in *P. falciparum*, roles of NtA remain unstudied. While NtA of exported proteins was described in 2008 [4], characterization of this role has been stymied by lack of information on which NAT(s) may be involved in this process, as well as a lack of tools for genetically manipulating large numbers of candidate proteins. Recent advances in methods for manipulating the parasite genome have now put these questions within reach.

By sequence analysis, *P. falciparum* has eight candidate N-acetyltransferases. Two are not transcribed during asexual replication in RBCs and therefore likely play no role in protein export. Of the remaining six (**Table 1),** three are clear orthologs of conserved eukaryotic NATs, Naa10, Naa20, and Naa30. Although no *P. falciparum* NAT has a hydrophobic signal sequence for ER translocation, Pf3D7_1437000 has a transmembrane domain. ER trafficking is incompletely understood in *P. falciparum*, and some proteins that lack both a canonical signal peptide and a transmembrane domain still traffic to the ER [8]. As such, one or more of the remaining five candidates may be ER-resident and may play a role in acetylating exported proteins. Here, we will use recently described reverse genetics methods to characterize the localization and function of the unknown *P. falciparum* NATs. In doing so, we will address the long-standing question of the role of NtA in protein export, as well as build a foundation for further study of the roles of NATs in *P. falciparum* biology. NATs found here could serve as anti-*Plasmodium* drug targets in the future, or may help us uncover other druggable targets in the essential path of protein export.

Name	Ortholog	Prelim. Localization	Effect of Knockdown
Pf3D7_1003300	Naa10	**Determine in Aim 1**	
Pf3D7_0109500	Naa20		
Pf3D7_0805400	Naa30		
Pf3D7_0629000	-		
Pf3D7_1323300	-		
Pf3D7_1437000	-	ER	**Aim 2**

Table 1 – The six *P. falciparum* NATs by sequence analysis. Localization of each will determined in Aim 1, with ER-localized NATs being evaluated in Aim 2.

3. Preliminary Studies

To explore the role of the NAT Pf3D7_1437000 in *P. falciparum*, we used CRISPR-Cas9 mediated genome editing to integrate a 3x-HA tag to the C-terminus. Immunofluorescence assays using this tag indicate that Pf3D7_1437000 colocalizes with ER-resident plasmepsin V, suggesting it may reside in the ER (**Fig. 3**). This raises critical questions which we will address with the work proposed here:

(a) Is Pf3D7_1437000 essential for parasite survival and for protein export?

(b) Are other *P. falciparum* NATs ER-resident, and do these play a role in protein export or other processes?

Our group has a published record of using recently-developed genetic tools to target *P. falciparum* genes and interrogate their function [5,13]. As such, we have all necessary tools and expertise to carry out the proposed work. Additionally, in unpublished work we have been able to tag and knock down a panel of 12 exported proteins, showing our ability to work through larger sets of poorly characterized proteins within the challenging system of the malaria parasite.

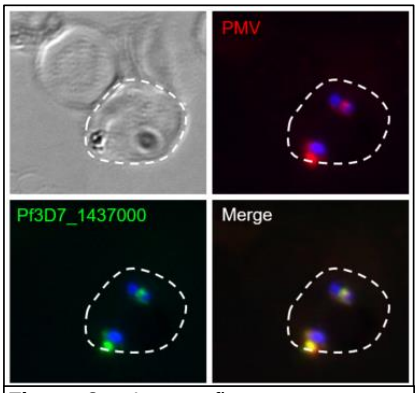

Figure 3 – Immunofluorescence assay showing colocalization of the ER-resident aspartic protease PMV with the candidate NAT Pf3D7_1437000-3xHA. Infected RBCs outlined with white dotted line. Red, mouse anti-PMV. Green, rabbit anti-HA. Blue, DAPI (nuclei).

4. Research Design and Methods:

Aim 1: Identify ER-resident NAT(s)

Our preliminary studies suggest that Pf3D7_1437000 localizes to the ER. However, other NATs may also reside in the ER to play redundant or unique roles in protein export or other processes. Here, we aim to address this critical question by endogenously tagging candidate NATs to determine which are resident to the ER. To accomplish this, the C-terminus of four of the six candidate NATs will be edited using CRISPR-Cas9 genome editing to incorporate the sequence for the fluorescent fusion protein mNeonGreen followed by a 3x-HA tag before the stop codon (**Fig. 4**). Two of the six candidate NATs (Pf3D7_1323300 and Pf3D7_0105900) have no viable Cas9 guide sites near the C-terminus, due to Cas9's requirement for an NGG adjacent to each target site. Instead these will be targeted using the recently-described AsCpf1 system which requires an NTTT, and has been validated in the literature in human cells [14], and in our lab for *P. falciparum*. mNeonGreen is a recently-described fluorescent protein with an extraordinarily high quantum yield of >0.8, making it substantially brighter than GFP, and allowing us to visualize even lowly-expressed proteins [15]. Localization of each NAT will be done in conjunction with endogenous tagging of the C-terminus of the ER marker BiP with mRuby3-3x FLAG. This system has been validated in our group previously for tagging of various proteins of interest.

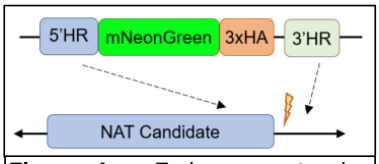

Figure 4 – Endogenous tagging strategy for NAT candidates. Each candidate is disrupted using Cas9 or Cpf1 immediately after the stop codon (yellow bolt). mNeonGreen and 3xHA are inserted by homologous recombination with flanking homology regions (HR).

Upon successful transfection, each NAT candidate will be screened by western blot to ensure that the C-terminal tag is intact. In any cases where the tags are removed, we will attempt to tag the NAT candidate using an N-terminal fusion. Once tags are verified, we will use fluorescence microscopy to assess the localization of each NAT-mNeonGreen in live cells. In each case, NAT-mNeonGreen parasites will be scored as having either ER localization (colocalization with BiP-mRuby3), nuclear localization (colocalization with DAPI, suggesting a misannotated histone acetyltransferase), cytosolic localization (dispersed signal through parasite cytosol), or "other" (a specific localization not consistent with the ER or nucleus). The function of NATs with a predominantly ER localization will be interrogated in Aim 2. Localization of non-ER NATs will be used to update current annotations and serve as a basis for future study of the role of NtA in *P. falciparum*.

Aim 2: Assess the role of ER-resident NAT(s) in parasite survival and protein export

Investigation of the role of NtA in the parasite ER has been hindered by lack of identification of the responsible ER-resident NAT(s). Here we will use the information from our preliminary work and Aim 1 to answer the critical questions of whether ER-resident NATs are essential for parasite survival and whether they are involved in protein export.

(A) *Are ER-resident NAT(s) essential for parasite survival?* To assess the role of Pf3D7_1437000 and other ER-resident NATs in parasite survival in RBCs, we will use CRISPR-Cas9 mediated editing as above to insert the recently-described TetR-DOZI aptamer knockdown system [16] which has been used successfully in our lab previously [13]. Briefly, a sequence is installed just after the stop codon which, when transcribed, folds into a 10x array of aptamers evolved to bind the Tet repressor (TetR). In the presence of anhydrotetracycline (aTc), TetR (fused here to the RNA helicase DOZI) is prevented from binding the aptamers. When aTc is removed, TetR-DOZI binds the aptamers, interfering with translation of the mRNA into new protein. Here we will use a variant of the previously published system developed in conjunction with the Niles lab at MIT which allows us to simultaneously install aptamers at the 5' and 3' ends of the gene (giving us more robust regulation) as well as flank the gene with loxP sites for inducible knockout (**Fig. 5**).

After successful transfection is verified, cultures will be split +/- aTc and the effect on the NAT level will be assessed by western blot to detect the 3xHA tag, while the effect on parasite growth will be assessed by monitoring growth curves using flow cytometry (using our group's BD Facs Canto). If knockdown of the given NAT candidate has no effect on parasite growth, we will use the DiCre-loxP system to induce knockout in the presence of rapamycin. If the NAT is not essential for parasite growth, the work outlined below will be done using the parasite line with the given NAT deleted.

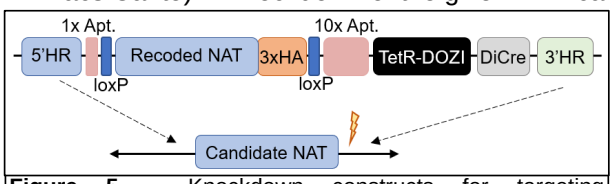

Figure 5 – Knockdown constructs for targeting Pf3D7_1437000 with aptamers for inducible knockdown. HR, homologous region.

(B) *Do ER resident NAT(s) have a role in protein export?* To test the role of each ER-resident NAT in protein export, we will first assess protein export in NAT-depleted parasites by immunofluorescence assays (IFA) (previously performed by our lab in [5]). Briefly, we will grow cultures with or without aTc for at least 24 hours, fix, and stain using antibodies against PEXEL-containing proteins (KAHRP and REX3) as well as a PEXEL-negative exported proteins (MSRP6) to test the degree to which NAT knockdown affects various exported proteins. For each IFA, at least 100 cells will be scored as "exported" (signal in the RBC cytosol >75% as bright as non-depleted cells) or "export impeded" (signal in RBC cytosol <75% as bright as non-depleted cells) and the mean cells exported will be compared using a Student's T test.

To determine if any export defect (or lack thereof) corresponds to a decrease in acetylation of exported proteins, exported proteins will be interrogated by mass spectrometry using an isobaric tagging approach. Here, cells will be grown in media with or without aTc, to allow for knockdown. For each knockdown, the two parasite cultures will then be lysed and exported proteins pulled down by immunoprecipitation using the antibodies mentioned above. Immunoprecipitated peptides will be digested with trypsin, then tagged using isobaric tagging reagents for relative and absolute quantification (iTRAQ), which covalently attach to amines (in this case lysine side chains which in each of the above proteins are present in the most N-terminal tryptic peptide) [17]. Samples will then be pooled and analyzed by liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS). During fractionation in the mass spectrometer, each iTRAQ tag produces a low mass ion unique to the sample it came from. These can be compared to determine relative abundances of a given peptide. If a given NAT is not involved in acetylating exported proteins, then we expect to see a ratio of iTRAQ low mass ions of approximately 1:1 for the N-terminal peptides of exported proteins. If a given NAT is involved in acetylating exported proteins, then we expect to see fewer acetylated N-terminal peptides in the presence of knockdown (i.e. a ratio of iTRAQ low mass ions <1 for the knockdown relative to the sample with aTc). This will allow us to determine if any of the ER-resident NATs identified in this proposal are responsible for acetylation of exported proteins.

5. Ethical aspects of the proposed research:

The work proposed above involves the use of both recombinant DNA and human erythrocytes for culture of *P. falciparum*. The Goldberg laboratory routinely manages both of these in nearly all of our work, and has specific plans in place for the safe use and disposal of recombinant DNA and human blood products. Human blood is generously provided by the blood bank at Barnes-Jewish Hospital, and is deidentified before receipt. As part of my regular laboratory work, I have completed compliance training through Washington University's office of Environmental Health and Safety for laboratory safety and waste disposal, as well as working with blood-borne pathogens.

12. Supporting Documentation

12.1. Referent 1 (Documents)

Name :	JohnParker
Position Title :	Associate Professor of Virology
Institution :	Cornell Univeristy College of Veterinary Medicine
Document Name 1 :	
Status 1 :	Not Uploaded

12.2. Referent 2 (Documents)

Name :	DavidSibley
Position Title :	Professor
Institution :	Washington University School of Medicine
Document Name 1 :	
Status 1 :	Not Uploaded

12.3. Referent 3 (Documents)

Name :	JamesFleckenstein
Position Title :	Associate Professor
Institution :	Washington University School of Medicine
Document Name 1 :	
Status 1 :	Not Uploaded

12.4. Sponsor 1 (Documents)

Name :	DanielGoldberg
Position Title :	Professor
Institution :	Washington University, School of Medicine

Document Name 1 :	Sponsor Biosketch AHA17.pdf
Status 1 :	Uploaded

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SPONSOR'S BIOGRAPHICAL SKETCH/BIBLIOGRAPHY

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Goldberg, Daniel Eliot	Professor of Medicine and Molecular Microbiology
eRA COMMONS USER NAME (credential, e.g., agency login)	
DANGOLDBERG	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Harvard College, Cambridge, MA	AB	1978	Biochemistry
Washington University, St. Louis, MO	MD, PhD	1985	Medicine, Biochemistry
Brigham and Women's Hospital, Boston, MA		1987	Internal Medicine
Washington University, St. Louis, MO		1988	Infectious Diseases
Rockefeller University, New York, NY		1990	Biochemical Research

PERSONAL STATEMENT:

I am a physician scientist with extensive experience studying the biochemistry and cell biology of Plasmodium falciparum. My lab has identified and contributed to our understanding of the biological roles of proteases in malaria parasite metabolism. It has studied the biology of hemoglobin degradation, amino acid utilization and heme metabolism in detail. The lab has made important contributions to our understanding of protein export in Plasmodium. It has also contributed a number of genetic tools for the study of this organism. Recently we have contributed to the antimalarial drug development effort. I have almost 30 years of experience mentoring students, postdocs and junior faculty. I was PI of the MSTP T32 training grant and am currently PI of the Infectious Diseases T32 training grant. Most of my trainees have gone on to independent academic positions. I have authored over one hundred peer reviewed papers and have two R01s from the NIH. I am on several major advisory boards, have been on more than 140 doctoral thesis committees and serve on a number of postdoctoral and junior faculty career development committees.

a. Russo I, Babbitt S, Muralidharan V, Butler T, Oksman A, Goldberg DE: Plasmepsin V licenses *Plasmodium* proteins for export into the host erythrocyte; Nature 2010, 463: 632-636.
b. Sigala PA, Crowley JR, Henderson JP, Goldberg DE: Deconvoluting heme biosynthesis to target blood-stage malaria parasites. eLife 2015, 4: e09143.
c. Beck J, Muralidharan V, Oksman A, Goldberg DE: PTEX component Hsp101 mediates export of diverse malaria effectors into host erythrocytes. Nature 2014, 511: 592-595.
d. Nasamu AS, Glushakova S, Russo I, Vaupel B, Oksman A, Kim AS, Fremont D, Tolia N, Beck JR, Meyers MJ, Niles JC, Zimmerberg J, Goldberg DE: Plasmepsins IX and X are essential and druggable mediators of egress and invasion in malaria parasites, Science 2017, in press.

APPOINTMENTS:

1978-79	Research Assistant, Department of Biological Chemistry, Harvard Medical School (laboratory of Dr. Eugene Kennedy)
1990-95	Assistant Professor of Medicine, Division of Infectious Diseases, and Assistant Professor of Molecular Microbiology, Washington University School of Medicine
1994-97	Assistant Investigator, Howard Hughes Medical Institute
1995-97	Associate Professor, Washington University
1998-2002	Associate Investigator, Howard Hughes Medical Institute
2002-2014	Investigator, Howard Hughes Medical Institute
1998-	Professor, Washington University
2000-	Co-Chief, Division of Infectious Diseases, Washington University

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HONORS:

1992	Charles Culpeper Scholarship in Medical Science
1995	Election, American Society for Clinical Investigation
1995-1997	Organizer, Molecular Parasitology Meeting, Wood's Hole, MA
1997	Burroughs Wellcome Molecular Parasitology Scholar Award
1999	Plenary Speaker, Danforth Symposium: Medicine at the Millennium, St. Louis
2000	Election, Fellow of the American Association for the Advancement of Science
2000	Election, American Association of Physicians
2002	Chair, Target Selection and Drug Development session, Keystone Symposium, Drugs against Tropical Protozoan Parasites, Keystone CO
2002	Chair, Microbial Proteases session, Proteolytic Enzymes Gordon Conference, NH
2004	University Lecture, University of Texas, Southwestern
2004	Keynote Address, Seattle Protozoology Conference
2007	Organizer, Keystone Symposium, Drugs Against Protozoan Parasites: Target Selection, Structural Biology and Medicinal Chemistry, Tahoe, CA
2007	Translational Medicine Annual Research Lecture, Albert Einstein College of Medicine, NY
2008	Outstanding Faculty Mentor Award, Washington University Graduate Student Senate
2011	Honors Program Lecture, New York University School of Medicine
2011	Pasteur Foundation Visiting Scholar Lecture, Institut Pasteur, Paris
2012	Plenary Speaker, BioMalPar meeting, Heidelburg, Germany
2012	Ricketts Symposium Lectureship, University of Chicago
2013	Washington University School of Medicine Distinguished Educator Award
2013	Washington University Second Century Award
2013	CC and Alice Wang Award, American Society for Biochemistry and Molecular Biology
2014	Washington University Medical Center Alumni Association Faculty Achievement Award
2014	Election, Fellow of the American Academy of Microbiology
2015	David M. and Paula L. Kipnis Distinguished Professorship
2017	Research Exemplar Project Award for Leadership and Integrity

SELECTED RESPONSIBILITIES:

1997-2007	Director, Medical Scientist Training Program
1997-2000	NIAID study section, Tropical Medicine and Parasitology
2000	Chair, NIAID Review Panel, Joint Ventures in Biomedicine and Biotechnology
2003	Chair, NIAID Special Emphasis Panel
2004-2009	Editorial Board, J Biol Chem
2006-2009	Section Editor, PLoS Pathogens
2005-2010	Umeå Centre for Microbial Research Scientific Advisory Board
2007-2011	Co-Director, Marine Biological Laboratories Course on Biology of Parasitism
2009, 2013	Advisor, NIAID Board of Scientific Counselors
2010	Member, Harvard Division of Medical Sciences Visiting Committee
2010	NIH Director's Distinguished Editorial Panel
2000-	Co-Chief, Division of Infectious Diseases
2003-	Johns Hopkins Malaria Research Institute External Advisory Board
2011-2014	Panelist, HHMI Medical Research Fellows Program
2011-2015	Member, NIAID Pathogenic Eukaryotes Study Section, Chair 2012-2014
2011-2017	Burroughs Wellcome Infectious Diseases Advisory Committee

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2014-2019	Member, NIAID Board of Scientific Counselors
2015-2020	Expert Scientific Advisory Council, Medicines for Malaria Venture, Geneva

CONTRIBUTION TO SCIENCE:

1. My early independent work focused on the oxygen-avid hemoglobin of the parasitic worm Ascaris. This is an abundant protein of the perienteric space. How and why it binds oxygen four orders of magnitude more tightly than human hemoglobin was unknown. We cloned the gene and showed that it has an ancestral plant-like origin. We determined that it has a tyrosine that protrudes into the binding pocket, forming a network of hydrogen bonds that grips molecular oxygen securely. We went on to show that it has an NADPH-dependent enzyme activity, and in collaboration with Jonathan Stamler, showed the it functions in reacting oxygen with nitric oxide to lower the oxygen concentration to which the microaerophilic worms are exposed.

a. Sherman DR, Kloek AP, Krishnan BR, Guinn B, Goldberg DE: The *Ascaris* hemoglobin gene: Plant-like structure reflects the ancestral globin gene. Proc Natl Acad Sci USA 1992; 89: 11696-11700.
b. Sherman DR, Guinn B, Perdok MM, Goldberg DE: Components of sterol biosynthesis assembled on the oxygen-avid hemoglobin of *Ascaris*. Science 1992; 258: 1930-1932
c. Kloek AP, Yang J, Mathews FS, Frieden C, Goldberg DE: The B10 tyrosine hydroxyl is crucial for oxygen avidity of *Ascaris* hemoglobin. J Biol Chem 1994; 269: 2377-2379.
d. Minning DM, Gow AJ, Bonaventura J, Braun R, Dewhirst M, Goldberg DE, Stamler J: *Ascaris* hemoglobin: a nitric oxide-activated deoxygenase. Nature 1999; 401: 497-502.

2. We have a long-standing interest in amino acid acquisition by intraerythrocytic malaria parasites. My laboratory has discovered and characterized a number of novel enzymes that participate in hemoglobin degradation, including plasmepsins, falcilysin and diaminopeptidase-1. We dissected the enzymological and biological roles of these proteins and proposed a semi-ordered pathway of hemoglobin degradation. We determined the biosynthesis, targeting and processing of these enzymes. Our further work showed that isoleucine is the only exogenous amino acid that is strictly required for parasite growth and we have found ways to exploit this dependence for chemotherapy.

a. Francis SE, Gluzman IY, Oksman A, Knickerbocker A, Mueller R, Bryant ML, Sherman DR, Russell DG, Goldberg DE: Molecular characterization and inhibition of a *Plasmodium falciparum* aspartic hemoglobinase. EMBO J 1994; 13: 306-317.
b. Gluzman IY, Francis SE, Oksman A, Smith CE, Duffin KL, Goldberg DE: Order and specificity in the *Plasmodium falciparum* hemoglobin degradation pathway. J Clin Invest 1994; 93: 1602-1608.
c. Banerjee R, Beatty W, Pelosof L, Klemba M, Goldberg DE: Four plasmepsins are active in the *Plasmodium falciparum* food vacuole, including a novel protease with an active site histidine. Proc Natl Acad Sci USA 2002; 99: 990-995
d. Istvan ES, Dharia NV, Gluzman I, Winzeler EA, Goldberg DE: Validation of isoleucine utilization targets in *Plasmodium falciparum*; Proc Natl Acad Sci USA 2011, 108: 1627-32.

3. We studied the metabolism of the P. falciparum digestive vacuole where hemoglobin is degraded, releasing heme, which is sequestered as crystalline hemozoin. It was unclear whether or not heme sequestration is spontaneous or involves protein. We showed that the histidine-rich protein II is exported by parasites into the host erythrocyte, ingested with hemoglobin into the digestive vacuole and is then capable of forming hemozoin. Subsequently another protein, HDP, was found to be even better at sequestration (Rathore and colleagues, PLoS Pathogens 2008). We went on to show that hemozoin formation was blocked by chloroquine and other antimalarial quinolines, in a test tube assay and in live cells. Recently, we have shown that, despite all the heme generated by hemoglobin

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catabolism, the infected red blood cell has the capacity to make its own heme using mostly red blood cell enzymes. This can be exploited for a novel approach to chemotherapy.

a. Sullivan DJ Jr, Gluzman IY, Goldberg DE: *Plasmodium* hemozoin formation mediated by histidine-rich proteins. Science 1996; 271: 219-222.
b. Sullivan DJ Jr, Gluzman IY, Russell DG, Goldberg DE: On the mechanism of chloroquine's antimalarial action. Proc Natl Acad Sci USA 1996; 93: 11865-11870.
c. Sullivan DJ Jr, Matile H, Ridley RG, Goldberg DE: A common mechanism for blockade of heme polymerization by antimalarial quinolines. J Biol Chem 1998; 273:31103-31107.
d. Sigala PA, Crowley JR, Henderson JP, Goldberg DE: Deconvoluting heme biosynthesis to target blood-stage malaria parasites. eLife 2015, e09143.

4. We have studied the function of asparagine repeats in Plasmodium falciparum. One-quarter of this parasite's proteins contain long stretches of Asn (one has 83 consecutive Asns). We have found that these proteins do not provide a cellular function but may have an evolutionary role in generating new protein functional domains. We have discovered that P. falciparum has a special version of Hsp110 that prevents amyloidogenic aggregation of these Asn-rich domains. To study this and other essential proteins, we have developed a destabilization domain system for protein knockdown in Plasmodium.

a. Armstrong CA, Goldberg DE: An FKBP destabilization domain modulates protein levels in *Plasmodium falciparum*; Nature Methods 2007, 4: 1007-9.
b. Muralidharan V, Oksman A, Iwamoto M, Wandless TJ, Goldberg DE: Asparagine repeat function in a *Plasmodium falciparum* protein assessed with a regulatable fluorescence affinity tag; Proc Natl Acad Sci USA 2011, 108: 4411-16.
c. Muralidharan V, Oksman A, Goldberg DE: Heat shock protein 110 maintains the asparagine-rich proteome of *Plasmodium falciparum*; Nature Comm 2012, 3: 1310.
d. Muralidharan V, Goldberg DE: Asparagine repeats in Plasmodium falciparum proteins. Good for nothing? PLoS Pathogens 2013; 9: e1003488.

5. We have been interested in how proteins get exported by the parasite out into the host red blood cell and what they do once they get out. We (as well as the Cowman lab) identified plasmepsin V as the ER aspartic protease that recognizes the proein export element (PEXEL) on precursor proteins, cleaves and sends them through the secretory system for translocation into the host cell. We showed that this is a highly specific protease, is essential and is an exciting drug target. We (and the Crabb lab) showed that a protein complex that had been postulated to be involved in translocation at the parasite-host cell interface, is indeed essential for this function and serves to export soluble and membrane proteins, PEXEL-containing and PEXEL-negative proteins, and proteins going to various destinations in the infected erythrocyte. Recently, we have identified a role for the exported protein histidine-rich protein II in cerebral malaria.

a. Russo I, Babbitt S, Muralidharan V, Butler T, Oksman A, Goldberg DE: Plasmepsin V licenses Plasmodium proteins for export into the host erythrocyte; Nature 2010, 463: 632-636.
b. Beck J, Muralidharan V, Oksman A, Goldberg DE: PTEX component Hsp101 mediates export of diverse malaria effectors into host erythrocytes. Nature 2014, 511: 592-595.
c. Pal P, Daniels BP, Oksman A, Diamond M, Klein RS, Goldberg DE: *Plasmodium falciparum* histidine-rich protein II compromises brain endothelial barriers and may promote cerebral malaria pathogenesis; mBio, 7: e00617-16.
d. Spillman N, Beck J, Goldberg D: Protein export into malaria parasite-infected erythrocytes: mechanisms and functional consequences; Ann Rev Biochem 2015, 84: 813-841.
http://www.ncbi.nlm.nih.gov/sites/myncbi/daniel.goldberg.1/bibliography/43980644/public/?sort=date&direction=ascending

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Research Support:

ACTIVE

T32 AI007172-34 09/01/1979 – 8/31/2021
NIH/NIAID
Infectious Diseases/Basic Microbial Pathogenic Mechanisms Training Grant
Goal: Training of pre and postdoctoral fellows
Role: PI

OPP1054480 (E. Winzeler) 10/09/2012 – 10/31/2019
Gates Foundation
Target Discovery for Antimalarials
The goal of this project is to identify the target enzymes for Malaria Box antimalarial compounds.
Role: Co-Investigator

R01 AI103280-01 (A. Odom) 12/01/2012 -11/30/2017
NIH/NIAID
Fosmidomycin resistance in Plasmodium falciparum
The goal of this project is to understand why isoprenoids are essential to malaria parasites, by metabolic and genetic analysis of fosmidomycin-resistant parasite strains.
Role: Collaborator

R01 AI112508 07/01/2014 – 06/30/2019
NIH/NIAID
Identification of the antimalarial target of pepstatin esters
Goal: To identify the mechanism of pepstatin prodrug on P. falciparum.
Role: PI

R01 AI126909 05/12/2016-04/31/2021
NIH/NIAID
Pathogenesis of HRPII in cerebral malaria
Goal: to study the mechanism of HRPII-mediated endothelial barrier disruption.
Role: PI

COMPLETED (DURING LAST THREE YEARS)

R21 AI110712 02/06/2014 – 1/31/2016
NIH/NIAID
Role of PfHO-1 in intraerythrocytic P. falciparum development
Goal: To characterize the role of a heme-binding transcription factor in malaria parasite development.
Role: PI

R01 AI047798-13 07/01/2000 – 11/30/2015
NIH/NIAID
Plasmepsin V function in malaria parasites
Goal: To characterize the enzyme that processes exported proteins in Plasmodium falciparum.
Role: PI

Name :	DanielGoldberg

Position Title :	Professor
Institution :	Washington University, School of Medicine
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Status 1 :	Uploaded
Document Name 2 :	Sponsor's list of Trainees AHA 17.pdf
Status 2 :	Uploaded

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SPONSOR'S LIST OF PAST AND CURRENT TRAINEES

POSTDOCTORAL

Past / Current Trainee	Trainee Name (Where Training Occurred)	Postdoc Research Training Period	Prior Academic Degree(s)	Prior Academic Degree Year(s)	Prior Academic Degree Institution(s)	Title of Research Project	Current Position of Past Trainees / Source of Support of Current Trainees
Past	Muralid-haran, V	06-12	PhD	06	Rockefeller	Poly Asn proteins	Assistant Professor, Cell Biology, U of Georgia
Past	Mallari, J	09-15	PhD	09	UCSF	Metalloproteases, plasmepsin V	Assistant Professor, Chemistry and Biochemistry, Cal State San Bernardino
Past	Sigala, P	09-15	PhD	09	Stanford	Heme Metabolism	Assistant Professor, Biochemistry, U of Utah
Past	Spillman, N	12-16	PhD	12	Australian National University	Exported Proteins	CJ Martin Early Career Fellow, University of Melbourne
Past	Beck, J	13-17	PhD	12	UCLA	Export machinery	Assistant Professor, Biomedical Sciences, Iowa State

PREDOCTORAL

Past / Current Trainee	Trainee Name (Where Training Occurred)	Training Period (Degree)	Prior Academic Degree Institution(s)	Prior Academic Degree(s)	Prior Academic Degree Year(s)	Title of Research Project	Current Position of Past Trainees / Source of Support of Current Trainees

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Past / Current Trainee	Trainee Name (Where Training Occurred)	Training Period (Degree)	Prior Academic Degree Institution(s)	Prior Academic Degree(s)	Prior Academic Degree Year(s)	Title of Research Project	Current Position of Past Trainees / Source of Support of Current Trainees
Past	Babbitt, S	06- 12	Xavier	BA	05	Amino Acid Sensing	Postdoctoral Fellow, Biology, Washington U
Past	Butler, T	07- 14	Florida A & M	BA	06	Exported proteins of unknown function	Postdoctoral Fellow, NIH
Past	Pal, P	10-16	Florida State	BA	08	Histidine-Rich Protein II	Resident in Internal Medicine, Tulane
Present	Polino, A	16-present	Cornell	BA	16	Function of protein acetylation in export	T32
Present	Nasamu, S	16-present	MIT	BS	15	Function of plasmepsin X	R01

Dr. Goldberg has served on 144 thesis committees, 18 of which are active.
Dr. Goldberg has previously trained 14 pre- and 19 post-doctoral fellows. Selected five:
*David Sherman, PhD: Professor, Seattle Biomed and Department of Global Health, University of Washington
*David Sullivan, MD: Professor, Dept of Molecular Microbiology & Immunology, Johns Hopkins School of Public Health
*Choukri Ben Mamoun, PhD: Associate Professor, Division of Infectious Diseases, Yale
*Ritu Banerjee, MD, PhD: Associate Prof, Division of Pediatric Infectious Diseases, Vanderbilt
*Marisa Ponpuak, PhD: Assistant Prof, Department of Microbiology, Mahidol University

Name :	DanielGoldberg
Position Title :	Professor
Institution :	Washington University, School of Medicine

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SPONSOR'S TRAINING PLAN

Parasites have evolved many clever ways to infect their hosts and develop within them. Study of these processes at a molecular level should lead to treatment or prevention of parasitic infections that afflict most of humanity. It will also shed light on general principles of biochemistry and cell biology. The organism we are studying is *Plasmodium falciparum*, a protozoan parasite that causes malaria.

Most of the malaria parasite`s adaptations to intracellular survival are still biological mysteries. Indeed, nearly half of the *Plasmodium* proteome comprises proteins of unknown function. We are interested in defining the roles of such proteins using a combination of genetic and biochemical approaches.

We are particularly interested in proteases and exported proteins. The parasite exports several hundred effector proteins into its host erythrocyte. What are these proteins doing in the host cell and beyond? How do the proteins get out of the parasite? We are excited about several aspartic proteases called plasmepsins, for which we have identified roles in parasite egress and invasion, as well as in protein export.

Drug resistance is now a major problem. New drugs are desperately needed. Thousands of antimalarial compounds have been identified in chemical library screens but we don`t know their targets. We are using approaches such as whole genome sequencing of resistant mutants, allelic replacement and chemical genetics to define promising new drug targets. Our work involves a combination of biochemical, genetic, genomic, cell biological, and physiological approaches aimed at understanding the biology of this nefarious organism.

A subject of particular interest in the lab is how *Plasmodium* effector proteins get exported into the host erythrocyte. The process starts in the ER, where most exported proteins are recognized by plasmepsin V, an aspartic protease that recognizes a specific motif near the N terminus of exported proteins and cleaves after a conserved leucine. The new N terminus is then acetylated. Mutations in the P2 position prevent acetylation and interfere with protein export, but it is not at all clear that acetylation is required for export. Alex's project will be to find and study the N-acetyl transferase responsible for this post-translational modification, to gain insight into this important question.

I am fully invested in ensuring Alex's development into a well-trained scientist. I have been mentoring trainees for almost 30 years. My trainees have been highly successful at going on to careers in research. There is a rich programmatic, departmental and lab environment. The Department of Molecular Microbiology is a dynamic, interactive environment full of seminars, informal interactions, and a whole network of students that forms the backbone of collaborative research. The wealth of conferences provides opportunities for Alex to present his work and learn critical evaluation of the scientific literature. For Alex this includes:

-Participation in:

Infectious Diseases and Basic Microbial Mechanisms Conference (weekly)

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Parasitology Journal Club (biweekly)
Tropical Diseases Research Meeting (biweekly)
Goldberg/Sibley lab meeting (biweekly)
Goldberg lab data meeting (weekly)
Microbiology Program Retreat (yearly)
Molecular Parasitology Meeting, Woods Hole, MA (yearly)
Research ethics course (yearly)

-Attendance at:

Molecular Microbiology Seminar Series (weekly)

I give feedback to Alex after each presentation, regarding scientific content and presentation skills. His graduate thesis committee includes: Drs. David Sibley (expert in parasitology), Phyllis Hanson and Sergei Djuranovich (cell biology), and Tamara Doering (microbiology). The committee meets with Alex every 6 months. The chair of his thesis committee, Dr. Tamara Doering, is a very experienced student mentor. The molecular microbiology program and division of biology and biomedical sciences has an active career guidance program. A series of seminars and workshops are held to expose students to different dimensions of the practice of science from teaching to industry to policy to entrepreneurship. Alex has also participated in a grant-writing workshop. I have lunch with Alex and the other student in the lab on a monthly basis to discuss scientific and career issues. Everything is in place for Alex's development as a scientist, and he will be fully prepared for a career as an academic scientist which is his tentative goal. We have done an IDP and update it on a regular basis subject to ongoing discussions. I anticipate that the combination of the enriching environment, his perseverance and his great intellect will be a formula for Alex's success.

The Goldberg lab is also an interactive, supportive, collaborative microcosm. The lab has a spectrum of senior researchers, students and technicians with a wide range of expertise and an environment of sharing. Resources for Alex's research are adequate, thanks to an R01 grant on mechanisms of aspartic protease inhibitors and thanks to generous discretionary funds that will allow Alex to go in various directions with his project. I meet with Alex on a weekly basis (Monday at 2 PM) and am also available routinely for unscheduled discussions. Alex will be given increasing responsibilities including writing grants (such as this one), supervising rotating students and undergraduates, presenting his work at national meetings (he has recently presented at his first meeting and won an award for best short talk with poster), and writing manuscripts for publications. I see my role as advisory. I expect Alex to take responsibility for each of these tasks, as he takes ownership of his research. He will come to me for comments and suggestions on what he has planned or written. I will give him my opinion and then he must decide whether and how to incorporate it. Alex has already shown scientific maturation over the past year. He is skilled at determining appropriate controls, alternative interpretations and the next experiments to be done with a high degree of

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independence. At this point I would say that he functions at the level of a more senior student. It is anticipated that the training Alex is receiving will allow him to develop a deep understanding of the malaria field and its important issues, obtain skills in malaria biology, general approaches to science, career skills/ strategies and will give him the tools needed to go out on his own and set up an independent research effort after further training.

Alex is highly intelligent, serious about his work and determined. He designs his own experiments. He is great at interacting and networking to generate new ideas and acquire new techniques. Alex's enthusiasm is infectious. He has been in the lab for one year, and is already starting to write a paper on plasmepsin V as a drug target, which we hope to submit to mBio. Alex is one of the top students I have had in 27 years at Washington University. He is certain to be productive and is destined to be a major contributor to the scientific community.

Name :	DanielGoldberg

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The Goldberg Laboratory occupies approximately 2,500 sq feet within the Department of Molecular Microbiology in the McDonnell Pediatric Research Building at Washington University. Dr. Goldberg has an office of approximately 200 sq feet. The lab has bench and desk space for 12-15 research personnel, with rooms for tissue culture, fluorescence microscopy, equipment, freezers and radioactive work. The laboratory is a BL-2 facility with a biohazard plan for working with blood-borne pathogens and a specific plan for precautions and containment for working with *Plasmodium falciparum*.

The Department of Molecular Microbiology has shared common facilities which include: constant temperature rooms at 4°C and 37°C; photographic darkrooms, glasswashing and media preparation facilities, a machine shop, a computer manager, an automatic X-ray film processor; a phosphorimager; and various microscopes and cameras, including a Cytation automated imaging setup. The department has an imaging facility for EM and confocal microscopy. There is a protein production and analysis facility, which includes an Octet apparatus for bio-layer interferometry. Relevant facilities within the School of Medicine include a DNA microarray facility, an electronics shop, FACS services, a nextgen DNA sequencing facility, peptide and oligonucleotide synthesis facilities, a proteomics facility, a high throughput screening facility and hybridoma, MS and NMR facilities. The Genome Engineering facility performs CRISPR design, assembly and validation for gene knockout, knock-in, tagging, allelic replacement and conditional modified alleles. The proteomics facility is skilled at performing analysis of post-translational modifications and quantitative analysis of protein/peptide levels.

Dr. Goldberg's research is conducted in a highly collaborative environment, with a close relationship
between the Department of Molecular Microbiology, the Division of Infectious Diseases, the Division of
Pediatric Infectious Diseases, and the Department of Immunology and Pathology. All have major programs in microbial pathogenesis. Because of the close physical proximity and academic ties, there is extensive opportunity for cross-fertilization between different parasitology groups (Sibley, Beverley, Tolia, Odom, Pearce, Weil) as well as with mycology, virology and bacteriology groups. There are joint seminars, symposia and work-in-progress talks. The Goldberg lab has a joint lab meeting with David Sibley's (Toxoplasma) lab as well as the groups of two very talented junior malaria researchers, Niraj Tolia and Audrey Odom. This rich environment contributes greatly to the research effort.